



Optimizing monitoring of an endemic terrestrial salamander (*Speleomantes ambrosii*): comparing cost-effectiveness of different methods for abundance estimation

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Abstract

In order to correctly plan conservation and management of animal populations, it is fundamental to obtain reliable information on population abundance using cost-effective monitoring methods. In the case of small terrestrial vertebrates, capture–mark–recapture, removal sampling and counts of unmarked individuals have proven to be reliable techniques to estimate population abundance. In the present study, we applied a multinomial N-mixture modelling approach, performed through a double-observer sampling protocol, to estimate population size of the endemic terrestrial salamander *Speleomantes ambrosii*. We aimed at comparing cost-effectiveness of this protocol with those of other estimation methods (i.e., capture–mark–recapture and removal sampling), with particular attention to management and monitoring guidelines. The double-observer multinomial N-mixture protocol was the most cost-effective among the different methods: given its ease of application and cost-effectiveness, we encourage its employment for long-term monitoring and conservation plans of the near-threatened plethodontid salamander *Speleomantes ambrosii*.

Keywords *Speleomantes ambrosii* · N-mixture models · Cost-effectiveness · Abundance · Double observer

1 Introduction

In a context of “biodiversity crisis”, where amphibian populations are systematically declining worldwide (Stuart et al. 2004), the identification of less expensive monitoring methods for robust population estimates may promote investments into conservation actions. For this reason, it is essential to obtain reliable ecological and demographic information by correctly coordinating efforts and available resources to obtain accurate predictions, adequate monitoring plans, and effective conservation strategies (Williams et al. 2002; Griffiths et al. 2015). However, since it is unlikely that all individuals present within a population are detected during a sampling session (i.e., detection error is always present), accounting for detection probability is fundamental to obtain reliable estimates of population abundance (Schmidt 2003; Costa et al. 2020).

Capture–mark–recapture (CMR) or temporary removal sampling and related statistical methods have been widely used to estimate demographic parameters of terrestrial salamanders in their habitats, while accounting for imperfect detection (Salvidio and Pastorino 2002; Lunghi et al. 2019, 2020; Renet et al. 2019). The former consists in capturing a number of animals, marking them appropriately, releasing them back into the population, repeating this for subsequent sessions and then determining the ratio of marked to unmarked animals in the population, while in the latter, all animals captured are physically removed from the population, kept in a holding area, and released at the end of the study. However, these methods may be expensive in terms of time, materials, and sampling effort (Dodd 2016). The recent implementation of N-mixture models allows analysis of count data of unmarked animals without the need for temporal (i.e., space-for-time substitution; Kéry and Royle 2016) or spatial replication (i.e., time-for-space substitution; Costa et al. 2019, 2021). These models, despite some criticism regarding parameter identifiability, assumption violation, and overdispersion (e.g., Barker et al. 2018; Link et al. 2018; but see Kéry 2018 for a comprehensive and favorable analysis of N-mixture models), have proven to be reliable

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within a disparate range of applications, since they provide reliable estimates of abundance and other demographic parameters with reduced time and field effort (Ariefiandy et al. 2014; Ficetola et al. 2018; Broker et al. 2019; Costa et al. 2019).

In recent years, some studies applied multinomial N-mixture techniques, obtained within a multiple-observer protocol, for the estimation of population abundance of small cryptic terrestrial animals, such as amphibians and reptiles (Costa et al. 2020; Romano et al. 2021; Rosa et al. 2022). These studies, by comparing abundance estimates with other methods, such as removal sampling (Costa et al. 2020; Romano et al. 2021) or distance sampling (Rosa et al. 2022), showed that multinomial N-mixture models give reliable and robust estimates of population abundance, even when detection and density are relatively low. The reliability of this method, in underground habitats, has been validated by Costa et al. (2020), which compared this approach with a removal sampling protocol of three sessions on the congener *Speleomantes strinatii*, during the main activity period of the species (i.e., summer), demonstrating that during the population activity peak, a single visit is sufficient to approximate total population size and density. Therefore, we applied this multinomial N-mixture framework on the near-threatened cave salamander *Speleomantes ambrosii* in three underground environments, using a double-observer sampling protocol. The European plethodontid *Speleomantes ambrosii*, a fully terrestrial salamander endemic to a

limited area in NW Italy, is listed as Near Threatened (NT) by IUCN (2009), given that its range is less than 5000 km² and suitable habitat may be declining locally. The Ambrosi's cave salamander is found in humid underground habitats such as natural and artificial caves, but also in humid forest environments (e.g., leaf litter near streams and on wet rocky outcrops) along small streams (Lanza 2007). Given the reliability and time-effectiveness of the double-observer sampling protocol, we compared the cost-effectiveness of this protocol with those of removal, binomial N-mixture models and CMR methods. The evaluation of this methodology–cost relationship could help to define conservation strategies and prioritization of a poorly known and small-range species, by promoting the selection of accurate and precise estimators and using financial resources more efficiently.

2 Materials and methods

2.1 Survey design

The studied sites are three natural caves located in the Province of La Spezia (Liguria region, NW Italy; Fig. 1) that extend underground 15, 27, and 39 m, respectively. Each cave was divided using a metal tape ruler into 1-m linear sections and extending on both side walls and on the ceiling. These 1-m sections were used as spatial replicates (i.e., plots) for the application of multinomial N-mixture models.

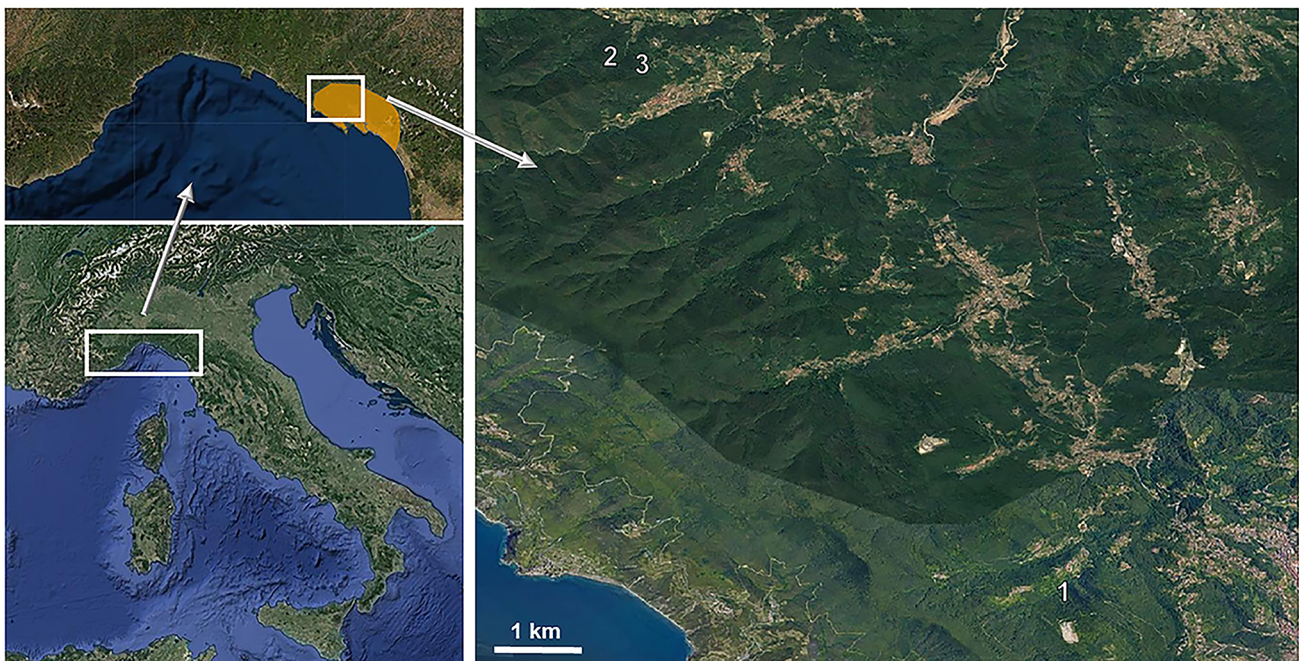


Fig. 1 Map showing species distribution range (orange area), provided by IUCN (2009), and location of the three sampling sites, across Northern Italy

Therefore, we sampled 15, 27, and 39 plots in Cave 1, 2, and 3, respectively. Since the multinomial protocol employed does not require multiple visits and salamanders did not move from one plot to another during sampling, we are not concerned about the demographic closure assumption and independence of sites (Royle 2004). We are not even concerned about the spreading of fungal infection, since, before entering the caves, we followed the procedures provided by Garner et al. (2016) for mitigating amphibian disease.

In September 2021, two experienced observers searched for salamanders with the aid of head lamps in all the 1-m plots, by applying a dependent-double-observer (DDO) method (Nichols et al. 2000). In the DDO method, a primary observer reports all the individuals counted to a secondary observer who records the data. In addition, the secondary observer takes records of any salamander that the primary observer failed to detect (Nichols et al. 2000). In the field, the two researchers alternated as primary and secondary observer. This protocol yields to three possible multinomial outcomes. An individual could be detected: (i) by the primary observer; (ii) by the secondary observer but not by the primary observer; (iii) by neither of the two observers (i.e., the quantity to estimate).

2.2 N-mixture modelling

Data were analyzed with a Poisson error distribution for the mean abundance at each sampling plot (λ) and considering constant detection probability across observers (Royle and Dorazio 2006). We modelled λ accounting also for the distance from the entrance of each cave, to test if animal distribution changed from the twilight zone to the inner parts of the underground environment. We selected the best between these two models (i.e., null model and model with the effect of distance from cave entrance) according to the Akaike's Information Criterion and considered that a $\Delta AICc > 4$ prove substantial differences (Burnham and Anderson 2002). We used a goodness-of-fit (GOF) test for model assessment. Total population estimates (N -hat) for each cave, along with 95% confidence intervals (CI), were obtained by posterior distribution of the latent abundance from the best model (Kery and Royle 2016). We conducted all statistical analyses in R with the package “unmarked”, through the function “gmultmix” (Fiske and Chandler 2011), and “AICcmodavg” (Mazerolle 2017) and built graphs using the package “forestplot” (Gordon and Lumley 2019).

2.3 Cost-effectiveness analysis

Finally, we provided a detailed estimate of costs for the application of this sampling protocol, compared with temporary removal and the CMR methods. In the case of removals, we considered three successive sampling occasions, this

being also the usual minimum requirement for the application of repeated counts on unmarked individuals in the framework of binomial N-mixture modelling to obtain reliable estimates (Ficetola et al. 2018; Costa et al. 2021). To attain the final costs, we calculated the expenses required for monitoring all the sites where the presence of the species is confirmed. We used the available data on Global Biodiversity Information Facility (GBIF) and the Ligurian Regional Cartographic Service. We considered both real costs such as researchers' remuneration, field trip, and equipment and survey costs, calculated as hours and days of working effort (Ariefiandy et al. 2014; Romano et al. 2017). The estimates of costs regarding the equipment (laser meter for measuring caves' plots during DDO monitoring, fauna boxes for storing animals during removal sampling and camera to photograph animals in a CMR study) were obtained by the mean of mid-range equipment costs from international online sellers, while the expenditure for researchers' remuneration and accommodation has been calculated on the base of the average international values (euros and dollars). Concerning data analyses, we did not consider the remuneration cost for researchers, but only estimated the level of complexity of each analysis, since time for data computerization and computation could be highly variable. Finally, we calculated the cost per kilometer during field trips on the basis of the Automobile Club Italia (ACI) parameters.

3 Results

We counted a total of 202, 7 and 34 salamanders, in Cave 1, 2, and 3, respectively. The best model for site abundance was that accounting for the distance from the entrance ($AICc = -338.92$ vs $AICc_{dist} = -363.54$), which had a significant negative effect on $\lambda = 0.602$; $SE = 0.120$; p value < 0.001). The GOF showed a good fit of the model with the data (p value $= 0.153$; c -hat $= 1.2$). Detection estimates were very similar among sites and all > 0.80 (Fig. 2). Population estimates from the best model were N -hat₁ = 207 (95% CI 185–231), N -hat₂ = 7 (95% CI 2–13), and N -hat₃ = 35 (95% CI 24–46). Plot abundance was also very different among sites (Table 1) with $\lambda_1 = 14$ (95% CI 12–16), $\lambda_2 = 0.27$ (95% CI 0.12–0.56), and $\lambda_3 = 0.89$ (95% CI 0.63–1.24).

The estimated total cost for the DDO method was 4624 €, while for removal sampling and CMR method was 6930 € and 8506 €, respectively (Table 2). The highest gap was related to the remuneration for research officers conducting field surveys, given the greater number of sampling days for removal sampling (20) and CMR (24) compared to DDO (7), while there was no difference related to sampling material.

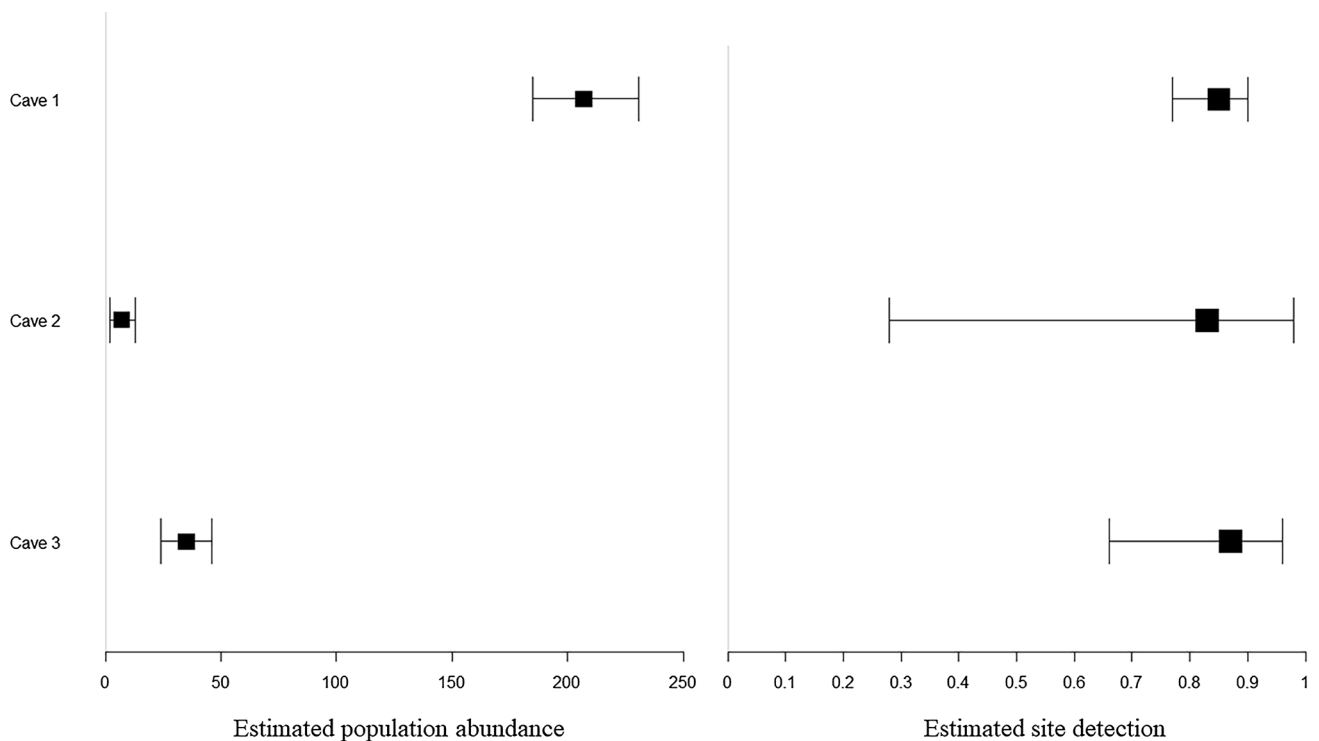


Fig. 2 Forest plot with total population estimates (left) and estimated site detections (right), along with 95% confidence intervals (horizontal bars) for the three sites

Table 1 Abundance and detection probability estimates together with 95% Confidence Intervals (CI), for the three subterranean sites

Site	Counts	Abundance \hat{N} (CI)	Plot abundance λ (CI)	Detection p (CI)
Cave 1	202	207 (185–231)	14 (12–16)	0.85 (0.77–0.89)
Cave 2	7	7 (2–13)	0.27 (0.12–0.56)	0.83 (0.28–0.98)
Cave 3	34	35 (24–46)	0.89 (0.63–1.24)	0.87 (0.66–0.96)

4 Discussion




Detection probabilities were high and very similar in the three study sites, despite the different estimated abundances (Table 1). These results resemble those obtained with other congeneric *Speleomantes* species, whose individual mean capture probabilities in subterranean environments are usually > 0.50 (Lindstrom et al. 2010; Lunghi et al. 2018; Costa et al. 2020).

The negative effect of cave depth on λ seems to be in good agreement with the results of previous studies on *Speleomantes* (Cimmaruta et al. 1999; Lunghi et al. 2015; Salvidio et al. 2020). In fact, the spatial distribution of salamanders along the cave is affected by the interaction between multiple environmental and social factors (e.g., light, humidity, temperature, and competition). Specifically, there is a clear age-class segregation, with juveniles living in more external cave sectors than adults which,

in turn, are dispersed along the inner parts of the cave, with high abundances in the intermediate sectors (Salvidio and Pastorino 2002; Ficetola et al. 2013; Salvidio et al. 2020). To better understand what influences population abundance and spatial distribution of the Ambrosi's cave salamander, it will be necessary to carry out further investigations, taking into account age classes and environmental factors.

The estimated field activity to evaluate the abundances of *Speleomantes ambrosii* populations within its distributional range, through the DDO approach, required only 7 working days for a two-person team, about a third less than removal sampling, binomial N -mixture models, and CMR. This difference is due to the characteristics of the different sampling protocols. In fact, to obtain reliable estimates through removal sampling, binomial N -mixture, or CMR, it is usually necessary to carry out at least three sampling sessions for each site, in contrast to the single session of the dependent double-observer multinomial N -mixture protocol

Table 2 List of costs and time required for the complete study for each sampling protocol**Monitoring***Field costs*

	DDO 		REMOVAL* 		CMR 	
	Person-hours	Sampling days	Person-hours	Sampling days	Person-hours	Sampling days
Survey of 6 sites	6	1	18	3	21	3
Survey of 40 sites	40	7	120	20	147	24
<i>Economic costs</i>						
	Sampling days	Cost/person * day ⁻¹	Sampling days	Cost/person * day ⁻¹	Sampling days	Cost/person * day ⁻¹
Remuneration for research officers conducting field surveys	7	228€ 234\$	20	228€ 234\$	24	228€ 234\$
Total	3192€ 3275\$		4560€ 4680\$		5472€ 5615\$	
<i>Travel</i>						
	Km/trip	Cost/km	Km/trip	Cost/km	Km/trip	Cost/km
Field trip	200	0.28€ 0.3\$	200	0.28€ 0.3\$	200	0.28€ 0.3\$
Total	1400	392€ 406\$	4000	1120€ 1150\$	4800	1344€ 1380\$
<i>Data analysis</i>						
Level of complexity (1–3)	1		1		2	
<i>Additional running and equipment costs</i>						
Sampling material	200€ 205\$		50€ 51\$		250€ 255\$	
	Laser meter		Fauna box		Camera	
	Head lamp		Gloves		Gloves	
			Permits		Permits	
Accommodation and food during surveys	60€-61\$/person * day ⁻¹		60€-61\$/person * day ⁻¹		60€-61\$/person * day ⁻¹	
Total	1040€ 1067\$	Study equipment and accommodation for two observers for 7 sampling days	1250€ 1283\$	Study equipment and accommodation for one observer for 20 sampling days	1690€ 1735\$	Study equipment and accommodation for 24 sampling days
Totals						
Working days	7		20		24	
Costs	4624€ 4745\$		6930€ 7111\$		8506€ 8730\$	

*Indicates that the same cost can be applied in the case of a repeated count *N*-mixture sampling protocol

used in this study (Ficetola et al. 2018; Costa et al. 2020). In any case, the correct application of the DDO sampling protocol should require a very good knowledge of the species seasonal activity pattern (e.g., with a pilot survey over the entire population activity period), because DDO has to be performed during the period of highest activity of the focal population. Since in the present study, we performed field sampling during the activity peak of the species, we are confident that our abundance estimates of the active

population are representative of the entire population, even if not accounting directly for availability, and our results are comparable with those obtained with removal sampling or CMR protocol, as reported by Costa et al. (2020) after comparing DO with a multi-day removal sampling on the congener *S. strinatii* on similar conditions. Furthermore, the manipulation of animals, required by methods that need individual marking or physical capture, implies the request for official capture and handling permits to be issued by

the Italian Ministry of Environment or local authorities, whose processing may take a long time, and a greater risk of spreading amphibians' pathogens, such as chytridiomycosis.

5 Conclusions

The benefits related to the application of the DDO sampling method in a hierarchical framework are several, since it is a reliable, quick, and economic protocol to estimate population abundance of amphibians without the need of handling individuals, preventing any possible negative effects on animals. Given the reliability, the easy application and the cost-effectiveness of this sampling protocol, we suggest that the employment of this sampling framework should be considered when planning conservation strategies or long-term monitoring of *Speleomantes ambrosii* and other terrestrial salamander species.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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